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FURTHER OBSERVATIONS ON THE ADAPTATION OF PARASITIC MICROORGANISMS TO A LOWERED OXYGEN TENSION *

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We have already published notes which tend to establish the generalization that many, if not all, endoparasites become adapted to a tension of oxygen below the atmospheric.[1] The following observations furnish further proof:

Partial-Tension Filaria.—Embryo filaria in the blood of a Carolina bullfrog survived for a number of days, at least 9, under partial oxygen tension, while they died within 24 hours under aerobic conditions. In the partial-tension culture there appeared a culture of flagellates resembling the cultural forms of a trypanosome, while no such organisms appeared in the aerobic culture.[2] We have suggested that this partial oxygen requirement is probably an important factor in the life history of many embryo nematodes, cestodes, and trematodes. It may, for example, help to explain why the embryos of Ankylostoma and Necator thrive best in a sandy soil.

Partial-Tension Herpetomonads.—We have made aerobic and partial-tension cultures (in the water of syneresis on slants of +0.5 Martin's medium containing pleuritic and ascitic fluid) of herpetomonads from the intestinal tract of the green bottle fly. The herpetomonads survived in an actively motile condition for 24 hours at 28 C., but after that the accompanying bacteria overgrew them entirely. The interesting point to record here is the relation of oxygen to the motility of the herpetomonads in these two different cultures: When mounted under a coverslip from the aerobic culture the herpetomonads remained motile only at the edges of the preparation or at the periphery of a bubble of air; in a similar preparation from the partial-tension culture the reverse obtained.

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[1] Jour. Infect. Dis., 1916, 19, p. 288.

[2] Lancet Clinic, 1916, 116, p. 1.

Partial-Tension Streptococcus from a Microaerophile-Aerophobe Strain.—The history of the isolation of the microaerophile-aerophobe strain was as follows: About 3 c.c. of prostatic fluid were "collected aseptically" from a case of low-grade prostatitis accompanied occasionally by mild attacks of sciatica. (It is not claimed here that there was any connection between the isolated organism and the condition of the patient. The organism might even have been a transient inhabitant of the urethra. In fact an attempt to isolate the same organism 2 weeks later failed.) The prostatic fluid was diluted with 10 c.c. of autoclaved distilled water and centrifugated at high speed for 30 minutes. The sediment was planted on slants of +0.5 Martin's medium, containing sterile ascites fluid, and incubated at 37 C., aerobically, at partial tension, and anaerobically. In 48 hours there was no aerobic growth (nor did any appear subsequently), while the partial-tension slant showed a number of very minute colonies, which a few days later attained the size and appearance of the colonies on the anaerobic slant. The anaerobic slant in 48 hours showed about 15 colonies, 3-4 mm. in diameter, composed of very long chains of streptococci.

One of the colonies on the anaerobic slant (which appeared pure macroscopically and microscopically) was subcultured to a tube of the same medium and incubated anaerobically at 37 C. Three days later the long chains in the water of syneresis of this anaerobic slant were thoroughly mixed with 10 c.c. of autoclaved salt solution and about 2 c.c. of this emulsion were planted with a sterile capillary pipet throughout the various portions of a deep column (10 cm.) of +0.5 Martin's medium containing an equal volume of sterile ascites fluid. After 24 hours' growth at 37 C. the tube presented the appearance shown in Fig. 1. That is, the growth was in 2 widely separated zones; an upper, 5 mm. broad, beginning about 1 mm. below the surface, and a deep zone about 10 mm. broad and sharply separated from the bottom of the test tube by a layer of colony-free agar 5 mm. high. The agar between the zones was entirely free of visible growth except one rather large colony as represented in Fig. 1. This tube maintained this appearance during 6 days' incubation at 37 C., except that some of the upper colonies in both zones increased in size, being comparatively isolated in distribution.

A similar phenomenon has been recorded by Wittneben.[3] While reading his article sometime previously, we were inclined to believe that Wittneben had been working with a mixed culture. It seemed extremely improbable to us that the culture of a single species, all the individuals of which were grown under the same conditions, could be composed of descendants adapted to such widely varying oxygen requirements. However, the following observations have reversed our preconceived ideas on this subject. But before we take up the study of the oxygen requirements of the cocci in these two widely separated layers of growth, we shall describe briefly the microscopic appearance of the colonies in the upper, and deeper layers. Some of the colonies appeared, under the hand lens, to be well defined and lenticular, like staphylococcus colonies, and when a mixture of these colonies was crushed under a coverglass, it was seen that some were composed of cocci in pairs and masses like staphylococci while most of the colonies were composed of streptococci. All of these retained the stain in Gram's method. The colonies in the deepest portion of the deep layer seemed to be composed entirely of long-chained streptococci, but one of the larger colonies near the upper limits of the deep layer was composed of cocci in chains, and pairs, and massed like staphylococci.

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[3] Centralbl. f. Bakteriol., I. O., 1907, 44, p. 96.

Now, as we did not encounter the staphylococcus arrangement in any of our subsequent subcultures on slanted media, whereas lenticular colonies frequently appeared among the others in the deep subcultures, we are inclined to believe that it will be worth while to investigate the variations in the mass grouping of bacteria, for this character may have been given too important a position in classification.

After the deep tube culture (Fig. 1) had been kept for 6 days at 37 C. the following studies were made:

(a) The upper layer: A block of colonies was cut out and ground up with sterile salt solution. A few drops of this suspension were allowed to flow over the surface of a slant of Martin's pleuritic medium and this was incubated at 37 C. after being attached to a freshly inoculated slant of B. subtilis. In 24 hours about 75 colonies, 0.25-2 mm. in diameter, were present on the surface, and the water of syneresis was filled with flocculi composed of long chains.

(b) The deep layer: After the tube had been cut at the level of the lowest colonies, a block was cut out at the point marked × (Fig. 1). This was planted in the same way as described under (a) except that here both partial-tension and anaerobic cultures were made. In 24 hours the growth on the partial-tension slant resembled that described under (a). The anaerobic colonies were larger, 2-4 mm.

Transplants were made from well-isolated colonies on each of these slants to slants of +0.5 Martin's pleuritic medium and incubated at 37 C. under the corresponding oxygen tensions.

Also, when the slant subcultures (mixed colony subcultures) described under (a) and (b), were 48 hours old, the growth in the water of syneresis was emulsified with sterile salt solution and planted in deep tubes of +0.5 Martin's medium containing an equal volume of ascites fluid. Of course in all such transfers separate sterile pipets were used. The anaerobic culture from the deep layer was designated (a), the partial-tension culture from the same (b), and the partial-tension from the upper layer (c). The results are shown in Figs. a, b, and c. That is, the anaerobic culture from the deep layer (a) yielded numerous relatively anaerobic colonies, 1-2 mm. in diameter, extending from 12 mm. below the surface to the bottom of the test tube; and extending from just beneath the surface to a depth of 5 mm. there were very numerous minute colonies. The growths in (b) and (c) were very much like those in (a), but the number of deep anaerobic colonies diminished progressively. During 7 days' incubation at 37 C. these growths remained much the same except that very fine colonies appeared throughout. No growth at any time occurred on the surface.

The single-colony transfers referred to under (b) were not tested for their oxygen relationships until after 7 days' incubation at 37 C. These were designated a′, b′, and c′ to correspond with a, b, and c. The growths in the water of syneresis were planted in the same way, the same batch of medium and the same batch of ascites fluid being used as in the case of a, b, and c. The results after 24 hours at 37 C. are shown in Figs. a′, b′, and c′. For example:

(a′) It will be noted that there were very few minute colonies near the surface; a layer of colonies 0.25 mm. in diameter, which was 6 mm. broad, extended from 2 mm. below the surface; then a space of 2 mm. free of colonies; then scattered large colonies, 1-2 mm. in diameter, extending to the bottom of the test tube, and between these a few smaller colonies.

(b′) Here there was a layer of very numerous minute colonies extending from just beneath the surface to a depth of 5 mm. The rest of the tube was free of growth, except for 6 scattered colonies 1-2 mm. in diameter and about a dozen scattered minute colonies.

(c′) This showed still further tendency to partial-tension growth in that only one deep colony, 0.5 mm. in diameter, developed.

Now we should like to remark right here that we entertained the idea that up to the point where Experiments a, b, and c were performed we might have been carrying over a mixed culture of a microaerophile and an aerophobe. But the single-colony subculture tests we think indicate that organisms in a pure culture may vary considerably and that if maintained at a certain tension for a sufficient length of time, most of the individuals tend to adapt their respiratory mechanism to that tension. That the transformation may have to be brought about gradually and may be accompanied by the development of a large number of related types, we think extremely probable. We also

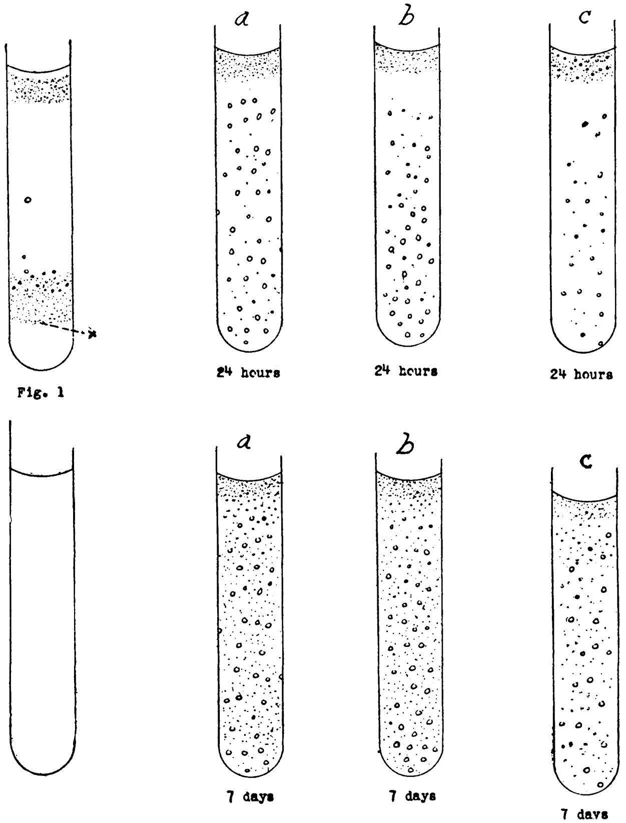


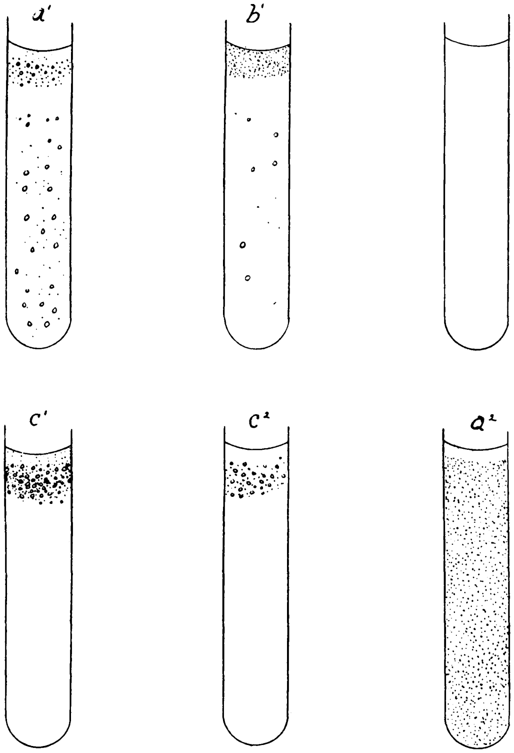
Fig. 1. A deep shake culture of the streptococcus, showing 2 widely separated zones of growth; an upper, or microaerophile zone, and a lower, or aerophobe, zone.

Fig. 2. A shake culture from an anaerobic subculture from X, Fig. 1.

Fig. b. A shake culture from a partial-tension subculture from X, Fig. 1.

Fig. c. A shake culture from a partial-tension subculture from the microaerophile zone (Fig. 1) at 24 hours and at 7 days. All shake cultures were made after growth for 48 hours at the corresponding oxygen-tension.

realize that our argument could have been strengthened by more extended experiments and that we should have tried to convert the microaerophile strain c′ into an aerophobe. In fact, this microaerophile strain did show aerophobe variants as shown in Figs. c and c′, altho c was derived from a culture carefully cut out of the upper layer of the original deep tube, Fig. 1. Furthermore, the 7-day-old tubes, a, b, and c, showed the slow development of a large number of minute colonies not present in 24-48 hours, and these appeared at all levels.



Figs. a′, b′, and c′. Shake cultures to correspond with a, b, and c, but made after pure-colony subcultures were maintained at the corresponding oxygen tensions for 7 days.

Fig. a^2. Result of attempting to make the aerophobe strain completely aerophobic.

Fig. c^2. Result of attempting to make microaerophile strains completely microaerophilic.

However, we did try to get a strictly aerophobe strain from a′ and a purely microaerophile one from c′ as follows: Single colonies were transplanted from the slants used to seed the deep tubes a′ and c′. These subcultures were designated a^2 and c^2. A^2 was transplanted and incubated under anaerobic conditions every day for 6 successive days. C^2 was kept at partial tension without transfer at the same temperature, 37 C., for the same length of time. Both of these were now tested with the results shown in Figs. a^2 and c^2.

At the same time that these culture tests, a^2 and c^2, were made, 2 animal inoculations were performed as follows: The slant cultures, a^2 and c^2, were suspended in sterile salt solution, sedimented, and resuspended in salt solution. Two half-grown rabbits each received 1.5 c.c. of these suspensions intravenously. The suspensions were approximately the density of a 24-hour typhoid broth culture. The rabbits were apparently well 48 hours later when they were killed. Both appeared normal on dissection except that the rabbit which had received the anaerobic strain, a^2, showed marked congestion of the tissues about both knee joints, which were full of a bloody fluid. Anaerobic cultures from this bloody exudate remained sterile.

The cocci had been grown on Martin's pleuritic or ascitic medium for exactly a month when this inoculation was performed.

While this single experiment does not definitely support the contentions of Rosenow regarding the relationship between localization in the body and oxygen tension, it does seem to point to the greater pathogenicity or toxicity of the aerophobe strain. At any rate the technic used here, and the observation that apparently more or less uniform adaptation can be brought about by maintenance at a certain tension, opens the way for further experiments.

SUMMARY AND CONCLUSIONS

Apparently animal as well as bacterial parasites are adapted to an oxygen tension below the atmospheric. The anaerobic culture of a streptococcus, when planted in a deep tube of agar, yielded widely separated zones of colonies, microaerophile and aerophobe. A further study of these two zones showed that both strains tended to throw off variants. Maintaining the microaerophile strain at partial-oxygen tension for several days produced a fairly uniform strain. Incomplete results were obtained by daily anaerobic transfers of the aerophobe strain. Each strain was inoculated into the circulation of a rabbit a month after isolation. The results are considered inconclusive, tho there were marked hemorrhages into the knee joints of the rabbit inoculated with the aerophobe strain.